

### AMENDMENTS TO THE SPECIFICATION

**Please replace the paragraph on page 48, lines 6-10 with the following paragraph:**

3 x 20 µl of these solutions were spotted on two aminated CDs and these CDs were incubated at 50 °C for 5 hours in a wet atmosphere. After three washes of 5 min with NaOH 0.4 N + ~~Tween~~ TWEEN® (Polyoxyethylene sorbitan monolaureate) 0.25% at 50 °C, these CDs were rinsed 3 times with water and dried at 37 °C for 30 min.

**Please replace the paragraph on page 48, lines 13-21 with the following paragraph:**

Both CDs were incubated 5 min in NaOH 0.2 N for denaturing capture probe, then rinsed with 0.1 M maleate buffer pH 7.5 with 0.15 M NaCl. These CDs were then incubated in a hybridization solution containing denatured DNA salmon sperm 100 µg/ml, SSC 4X, Denhardt 5X and denatured CMV biotinylated DNA at a concentration of 70 ng/ml for 2 hours at 65 °C. After hybridization step, the CDs were washed 3 times with 0.01 M maleate buffer containing 15 mM NaCl and ~~Tween~~ TWEEN® 0.3% at room temperature.

**Please replace the paragraph on page 48, lines 22-27 with the following paragraph:**

The first CD was then incubated with 0.1 M maleate buffer containing 0.15 M NaCl, 0.1% milk powder and streptavidin-peroxidase 1 µg/ml for 45 min at room temperature. After conjugates incubation, both CDs were washed 3 times with 0.01 M maleate buffer containing 15 mM NaCl and ~~Tween~~ TWEEN® 0.3% at room temperature.

**Please replace the paragraph on page 50, lines 6-15 with the following paragraph:**

20 µl of three different solutions of borate buffer 0.02 M NaCl pH 8.2 containing carbodiimide (Acros) at 1 mg/ml and one type of the three different antibodies at 10 µg/ml were spotted on three different pieces of CD. These spots were incubated overnight at 4 °C, and then rinsed for 10 min with glycine buffer 0.1 M pH 9.2 containing casein at 0.1%, then twice with glycine buffer 0.1 M pH 9.2 containing ~~Tween~~ TWEEN® 20 at 0.1% for 5 min and finally twice with glycine buffer 0.1 M pH 9.2. The CDs were dried at 37 °C during 30 min.

**Please replace the paragraph on page 50, lines 18-33 with the following paragraph:**

The CDs were incubated at room temperature with the three different antibodies bound onto the surface with a solution of serum albumin at 10 µg/ml in PBS containing 0.1% of casein. The incubation was for 90 min. The CDs were rinsed 3 times with PBS containing 0.1% of

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~~Tween~~ TWEEN® 20, and then incubated with biotinylated antibodies against serum albumin at 20 µg/ml in PBS containing 0.1% of casein for 45 min. They were then rinsed 3 times with PBS containing 0.1% of ~~Tween~~ TWEEN® 20, and then incubated for 45 min the CDs in a solution of PBS containing 0.1% of casein and either Streptavidin-peroxidase at 1 µg/ml. The CDs were rinsed 3 times with PBS containing 0.1% of ~~Tween~~ TWEEN® 20. For detection, the CD where streptavidin-peroxidase was fixed were incubated in a solution of TMB and pictures were taken after 2, 4 and 6 min under camera to see blue color appearing where antibodies against BSA and against streptavidin were spotted.

**Please replace the paragraph on page 51, lines 6-29 with the following paragraph:**

Streptavidin was diluted to a final concentration of 100µg/ml in a spotting buffer Borate 0.05M pH 8, glycérol 40 %, NONIDET P40 (NP40) (Octylphenolpoly(ethyleneglycolether) 0.02 % and spotted as an antigen at the surface of a polyacrylate based polymer coated CD. The CD contained layers of a r-CD (Recordable CD) which can be read and recorded by a laser beam directed on one side of the CD (the down part in a classical CD-reader) and the spotting was performed on the other side of the CD. The spotting was obtained with solid pins of 0.250 mm diameter and the spots were around 0.35 mm diameter final After 3 washes with phosphate pH 7.4 0.01M + 0.1% ~~Tween~~ TWEEN® 20, nonspecific binding sites were blocked with PBS containing milk powder at 0.1% for 1h at 20°C. The CD characteristics, mainly, the number of arrays, of spots and their identification were written on a part of the recordable : CD layers. Some parts were still free for further writing of the final detection results. For detection of antibodies, the CDs chambers were incubated for 1h at 20°C with rabbit anti-streptavidin ranging from 1.2 pmole to 12 amoles in 100µl in PBS + milk powder at 0.1%. After 4 washes of one minute with a 10 mM maleate buffer containing 15 mM NaCl and 0.1% ~~Tween~~ TWEEN® pH 7.5 ( washing buffer) CDs were incubated for 45 min at 20°C with a conjugate of anti-rabbit IgG/gold particles of 10 nm diameter (diluted 100 times) in 100 mM maleate buffer containing 150 mM NaCl.

**Please replace the paragraph on page 52, lines 6-17 with the following paragraph:**

Multiwell plates coated with streptavidin (Roche) were used as a support. The wells were incubated for 1h at 20°C with 100 µl of rabbit anti-streptavidin diluted from 1.2 pmole to 12 amole in 100µl in PBS with milk powder at 1/%.The wells were then washed 4 times with a 10

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mM maleate buffer containing 15 mM NaCl and 0.1% ~~Tween~~ TWEEN® pH 7.5 ( washing buffer).The plate were incubated for 45 min at 20°C with conjugate anti-rabbit IgG/peroxidase labeled diluted at 1/100 with 100 mM maleate buffer containing 150 mM NaCl. Wells were washed 5 times in the same washing buffer as before and then incubated for 10 min with TMB (in the dark). The reaction was stopped with 100µl of stop solution and the samples were ~~readed~~ read at 405 nm in a ELISA reader.

**Please replace the paragraph on page 56, lines 14-28 with the following paragraph:**

Clinical specimens (BAL, stumps, AL or ETA) were homogenized in 5 ml of TE buffer (20 mM Tris HCl, pH 8.0, 10 mM EDTA) containing 2% (w/v) SDS. The homogenate (1.5 ml) was then centrifugated for 5 min at 7500 xg. The cellular pellet was washed once with TE buffer, lysed in the presence of 1% (v/v) ~~Friton~~ TRITON® X-100 (Octylphenol-polyethylene glycol ether) and 50 µg of lysostaphin (Sigma Chemical Co., St. Louis, Mo), and incubated for 15 min at 37°C. Lysis was completed by adding 100 µg of proteinase K (Boehringer, Mannheim, Germany). The lysate was incubated for another 15 min at 55°C and 5 min at 95°C. It was centrifugated at 4000 xg for 5 min. In order to purify bacterial DNA, 200 µl of the supernatant were then filtered on a Nucleospin C+T column (Macherey-Nagel, Düren, Germany) washed and then eluted with 200 µl sterile H<sub>2</sub>O, according to the manufacturer's protocol. DNA suspensions were stored at -20°C.

**Please replace the paragraph on page 56, line 31 through page 57, line 12 with the following paragraph:**

Co-amplification by duplex PCR of fem-A and Mec-A genetic markers on Staphylococcus sample (was done using 2 consensus primers for fem A : Apcons31 : TAAYAAARTCACCAACATAYTC SEQ ID NO: 1), Apcons32 : TYMGNTCATTATGGAAGATAC (SEQ ID NO: 2), and 2 primers for mec A sequence: Apmec01:TCTGGAAGTTGTTGAGCAGAG (SEQ ID NO: 3) and Apmec02 : GGCTATCGTTGTCACAATCGTT (SEQ ID NO: 4) at a final concentration of 0.1µM each. The PCR was made in a Tris-HCl buffer 0.075M pH 9, KCl 50 mM, MgCl<sub>2</sub> 2 mM, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 20 mM buffer containing dATP, dCTP, dGTP, at a final concentration of 50 µM each and dTTP and dUTP-biotinylated (Roche, Indianapolis, USA) at a final concentration of 25 µM each. The

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PCR ran 5 min at 94°C, then 40 cycles made of 30 sec at 94°C, 45 sec at 49°C and 30 sec at 72°C, and finally 10 min at 72°C.

**Please replace the paragraph on page 57, line 23 through page 58, line 2 with the following paragraph:**

After hybridization, the chambers are removed and then the Bio-CD™ is washed 4 times 1 min with a maleate buffer 10 mM containing NaCl 15 mM and ~~Tween~~ TWEEN® 0.1% pH 7.5. The BioCD™ is incubated for 45 min at room temperature into a blocking buffer (maleate buffer 100 mM NaCl 150 mM pH 7.5 containing 0.4% caseine) containing a streptavidin-colloidal gold conjugate diluted 100 times (BBI, England). The BioCD™ is then washed 5 times in the same buffer (maleate buffer 10 mM NaCl 15 mM pH 7.5 ~~Tween~~ TWEEN® 0.1%). Then Bio-CD is incubated at room temperature for 15 min in the Silver Blue Solution (AAT, Namur, Belgium), rinsed in water, dried 5' at 37°C and read with the Bio-CD reader. Results are digitalized and quantified with softwares included in the workstation.

**Please replace the paragraph on page 64, line 25 through page 65, line 18 with the following paragraph:**

The clinical samples are first homogenized and bacterial lysed by lysostathin treatment. Typical methodology is the following for bacterial DNA extraction from clinical samples. Clinical specimens are homogenized in 5 ml of TE buffer (20 mM Tris HCl, pH 8.0, 10 mM EDTA) containing 2% (w/v) SDS (step 1). The homogenate (1.5 ml) is then centrifugated for 5 min at 7500 xg.(step 2) The cellular pellets are washed once with TE buffer, lysed in the presence of 1% (v/v) ~~Triton~~ TRITON® X-100 and 50 µg of lysostaphin (Sigma Chemical Co., St. Louis, Mo)(step 3) They are incubated for 15 min at 37°C. Lysis is completed by adding 100 µg of proteinase K (Boehringer, Mannheim, Germany)(step 4). The lysate is incubated for another 15 min at 55°C and 5 min at 95°C ( step 5). It is centrifugated at 4000 xg for 5 min (step 6). For further purification, bacterial DNA, the supernatant are then filtered through a chamber containing silica for binding DNA. The extract is introduced in the upper part of the chamber, go through the silica where the DNA binds (step 7). The other molecules are ~~washed~~ washed away (step 8). Then, water is introduced in the chamber to release DNA in solution (step 8). The DNA solution is then pushed into chamber 2 through a pipe after opening of the microvalve (step 9). In this example steps 7 to 9 are performed on the disc. It is also possible to adapt steps 1 to 6

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while changing the centrifugation steps into a filtration which can take place in a chamber present on the disc.

**Please replace the paragraph on page 68, lines 11-26 with the following paragraph:**

Streptavidin was diluted to a final concentration of 100µg/ml in a spotting buffer Borate 0.05M pH 8, glycérol 40 %, NONIDET P40 (NP40) (Octylphenolpoly(ethyleneglycolether)) 0.02 % and spotted as an antigen at the surface of a polyacrylate polymer coated disc. The spotting was obtained with solid pins of 0.250 mm diameter and the spots were around 0.35 mm diameter final After 3 washes with phosphate pH 7.4 0.01M + 0.1% ~~Tween~~ TWEEN® 20, nonspecific binding sites were blocked with PBS containing milk powder at 0.1% for 1h at 20°C. The discs were incubated for 1h at 20°C with rabbit anti-streptavidin ranging from 1.2 pmole to 12 amoles in 100 µl in PBS + milk powder at 0.1%. After 4 washes of one minute with a 10 mM maleate buffer containing 15 mM NaCl and 0.1% ~~Tween~~ TWEEN® pH 7.5 ( washing buffer) discs were incubated for 45 min at 20°C with a conjugate of anti-rabbit IgG/gold particles of 10 nm diameter (diluted 100 times) in 100 mM maleate buffer containing 150 mM NaCl.

**Please replace the paragraph on page 69, lines 2-14 with the following paragraph:**

Multiwell plates coated with streptavidin (Roche) were used as a support. The wells were incubated for 1h at 20°C with 100µl of rabbit anti-streptavidin diluted from 1.2 pmole to 12 amole in 100µl in PBS with milk powder at 1/%.The wells were then washed 4 times with a 10 mM maleate buffer containing 15 mM NaCl and 0.1% ~~Tween~~ TWEEN® pH 7.5 ( washing buffer).The plate were incubated for 45 min at 20°C with conjugate anti-rabbit IgG/peroxidase labeled diluted at 1/100 with 100 mM maleate buffer containing 150 mM NaCl. Wells were washed 5 times in the same washing buffer as before and then incubated for 10 min with TMB (in the dark). The reaction was stopped with 100µl of stop solution and the samples were readed at 405 nm in a ELISA reader.